Peer Review
Experimental Toxicology Division
NHEERL

CUMULATIVE RISK

DEVELOPMENT AND EVALUATION OF COMPONENT-BASED METHODS FOR INVESTIGATION OF INTERACTIONS

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ENVIRONMENTAL ISSUE

- Most risk assessments for chemical mixtures rely on component-based approaches, rather than data on the mixture itself, because mixture data are rarely available.
- Component-based methods use toxicological data on individual chemicals contained in the mixture. They may incorporate information on interactions, but this is rarely done.
- These methods allow assessment of health risk in the absence of mixtures data.
- The default assumptions underlying component-based methods have not been thoroughly evaluated and their ability to predict the effects of mixtures has rarely been evaluated by comparison of model predictions to experimental results.

OBJECTIVES/GOALS

- The purpose is development and evaluation of component-based methods for toxicological evaluation of interactions and risk assessment of mixtures.
- The principal project-level goal is development and refinement of efficient experimental designs (which require fewer treatment groups and animals than traditional full-factorial designs) for detection of departure from additivity and associated methods to predict the effects of chemical mixtures. We are developing, refining, evaluating and comparing 3 quantitative methods:

1) the use of single-chemical dose-response curves and an assumption of dose additivity (which requires an assumption of similar mode of action) to predict the effects of mixtures. This is the primary focus of the present poster.
2) estimation of higher-order mixtures toxicity from single-chemical and binary mixture data with the interaction-based Hazard Index.
3) proportional-response addition, a method that does not require mode-of-action assumptions, as mode of action information is frequently not available.

- Data-level goals for this project, include assessment of:

1) mixtures of all 4 regulated THMs (chloroform, CHCl3; bromodichloromethane, BDCM; chlorodibromomethane, CDBM; and bromoform, CHBr3) by comparison of observed hepatotoxicity to that predicted based on single-chemical data and assumptions of common mode of action and dose additivity.
2) the six binary combinations of the 4 regulated THMs by comparison of experimentally observed hepatotoxicity to that predicted based on single-chemical data and assumptions of common mode of action and dose additivity.
3) the effect of mixing ratio on interactive toxicity, comparing environmentally relevant mixing ratios to 1:1 mixing ratios.
4) the influence of dose on interactive toxicity.

- Hepatotoxicity was selected as the health endpoint because it was the health endpoint used to calculate 3 (CHCl3, CDBM, CHBr3) of the 4 THM oral Reference Doses.

EXPERIMENTAL DESIGN

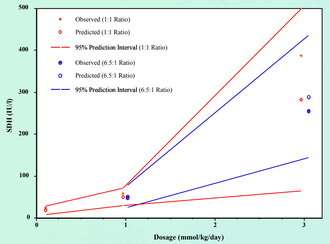
- Female CD-1 mice were exposed by gavage in an aqueous vehicle daily for 14 days. Solutions were made fresh daily in gas-tight vials.
- Hepatotoxicity was assessed on the 15th day by serum enzymes indicative of hepatic damage (SDH, ALT) and by liver histopathology. SDH is illustrated on this poster.
- Each experiment has 12 dose groups. These are:
  - 1 vehicle control group
  - 3 dose levels of THM 'A' alone
  - 3 dose levels of THM 'B' alone
  - 3 dose levels of A+B at a 1:1 mixing ratio
  - 2 dose levels of A+B at a mixing ratio based on THM levels in chlorinated drinking water (Krasner et al., 1989). The 'environmentally relevant' mixing ratios were different for each binary combination:

CHCl3:BDCM – 2.7:1	CHCl3:CDBM – 6.5:1
CHCl3:CHBr3 – 65:1	BDCM:CDBM – 2.4:1
BDCM:CHBr3 – 24:1	CDBM:CHBr3 – 10:1

- Statistical Analysis. Presented here are the results of the threshold dose-additivity model developed earlier in this project (Gennings et al. 1997, 1999). The dose-additivity definition is based on Berenbaum's interaction index: d1/Dx1 + d2/Dx2 + ... + dn/DXn = 1, with d1, d2 and dn the concentrations of the chemicals in the mixture and Dx1, Dx2 and Dxn effective (equally toxic) doses of each chemical (Berenbaum, 1985). The single-chemical dose-response curves are used to predict the mixture response. Biological variability is incorporated through calculation of prediction intervals. If the experimental response falls within the 95% prediction interval, the null hypothesis of dose additivity is not rejected.

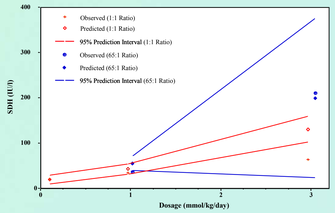
Mixing Ratio (CHCl3:BDCM)	Dosage (mmol/kg/day)	**Observed** Mean SDH (IU/l)	**Predicted** Mean SDH (IU/l)	95% Prediction Interval (IU/l)	p value Overall: 0.80
1:1	0.1	14.5	13.4	(6.6 - 20.3)	0.99
1:1	1.0	24.1	27.7	(13.5 - 41.8)	0.99
1:1	3.0	155.7	118.5	(17.5 - 219.6)	0.99
2.7:1	1.0	26.4	26.4	(12.1 - 40.8)	0.99
2.7:1	3.0	141.9	111.0	(41.7 - 180.2)	0.97

CHCl3: CDBM. Comparison of Observed (Experimental) Mixture Response to the Mixture Response Predicted from the Single Chemical Data Under a Hypothesis of Dose Additivity.



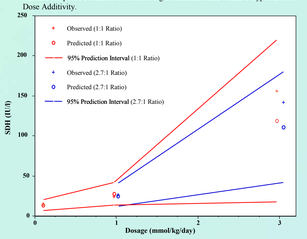
In each case, the observed (experimental) mean fell within the 95% Prediction Interval, indicating no detectable deviation from dose-additivity. These results are consistent with the overall test for additivity, p = 0.60.

CHCl3: CHBr3. Comparison of Observed (Experimental) Mixture Response to the Mixture Response Predicted from the Single Chemical Data Under a Hypothesis of Dose Additivity.



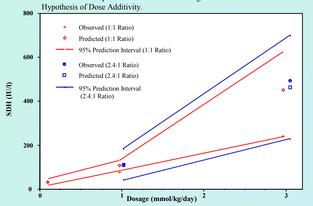
At the 1:1 mixing ratio, the observed (experimental) mean response for the 3.0 mmol/kg/day dosage fell well outside the 95% Prediction Interval, indicating less than dose-additive toxicity for this mixture point. The observed response at the 65:1 mixing ratio for the 1.0 mmol/kg/day dosage fell just below the 95% Prediction Interval, indicating less than dose-additive toxicity at this mixture point. The overall additivity test p-value was highly significant (p<0.001) with the degree of significance of the individual mixture point p-values consistent with the Prediction Interval assessments.

CHCl3:BDCM. Comparison of Observed (Experimental) Mixture Response to the Mixture Response Predicted From the Single Chemical Data Under a Hypothesis of Dose Additivity.



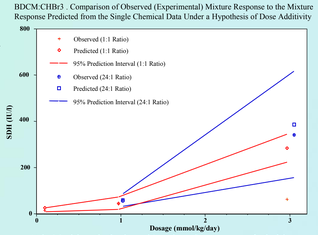
In each case, the experimental mean was close to the predicted mean and fell within the 95% Prediction Interval, indicating no detectable deviation from additivity. These results are consistent with the overall additivity test, p=0.80.

BDCM:CDBM. Comparison of Observed (Experimental) Mixture Response to the Mixture Response Predicted from the Single Chemical Data Under a Hypothesis of Dose Additivity.

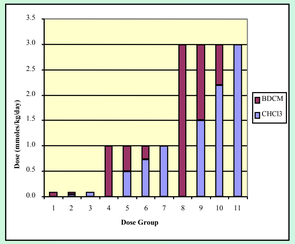


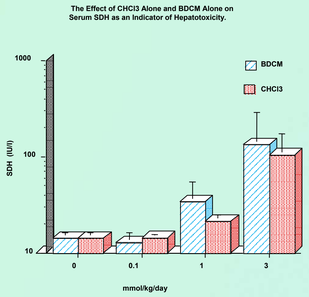
At the 1:1 mixing ratio, the observed (experimental) mean response for the 1.0 mmol/kg/day dosage was lower than the predicted mean response, falling barely outside the 95% Prediction Interval, indicating less than dose-additive toxicity for this mixture point. In all other cases, the observed mean fell within the 95% Prediction Interval, although just barely at 3.0 mmol/kg/day dosage, 1:1 mixing ratio, indicating no detectable deviation from dose-additivity. The overall additivity test p-value of 0.057, indicated, at most, borderline statistical significance and the individual mixture point p-values were consistent with the Prediction Interval assessments.

BDCM:CHBr3 . Comparison of Observed (Experimental) Mixture Response to the Mixture Response Predicted from the Single Chemical Data Under a Hypothesis of Dose Additivity



At the 1:1 mixing ratio, the observed (experimental) mean response for the 3.0 mmol/kg/day dosage fell well outside the 95% Prediction Interval, indicating less than dose-additive toxicity for this mixture point. The overall additivity test p-value was highly significant (p<0.001) with the degree of significance of the individual mixture point p-values consistent with the Prediction Interval assessments.





RESULTS/CONCLUSIONS

- For the 5 binary THM combinations analyzed to date, greater than additive (i.e. synergistic) interactions were not detected. The hepatotoxic interactions between these THMs were characterized as either dose-additive or less than additive (i.e. antagonistic).
- The influence of mixing ratio is seen in the interactions of CHBr3 with either CHCl3 or BDCM. At the 1:1 mixing ratio, less than additive toxicity was observed at the highest tested dose level, 3.0 mmol/kg/day, whereas the environmentally-relevant mixing ratios appeared dose additive.
- The influence of dose is seen at the 1:1 mixing ratio, when considering, again, the interactions of CHBr3 with either CHCl3 or BDCM. At 3.0 mmol/kg/day of either binary combination, there was clear evidence of antagonism, but not at the lower dose levels (1.0 and 0.1 mmol/kg/day) which appeared dose-additive.
- In sum, these binary combination data provide consistent evidence that the hepatotoxic interactions between the THMs are either dose-additive or antagonistic, with the nature of the interaction (additivity, antagonism) dependent on the chemicals in the mixture, their dose levels and mixing ratios.

IMPACT

- This research is directly responsive to the mandate of the Safe Drinking Water Act Amendments of 1996 requiring EPA to develop new approaches to the study of mixtures found in drinking water.
- Useful methodology to predict the effects of mixtures from single chemical data has been developed and implemented. This method assumes dose-additivity which has an underlying assumption of similar mode of action.
- The methodology developed by this group has been adopted for use by other investigators both within and outside EPA. Chemical mixtures that have been or are currently being evaluated include: metals (a 4-chemical mixture), pesticides (4- and 5-chemical mixtures); haloacetic acids (a 9-chemical mixture and various sub-sets of these 9 chemicals); and, endocrine disruptors (an 18-chemical mixture).
- A data set has been developed that is being used to develop, refine and assess 3 quantitative methods. Analysis of the same data by the 3 methods may facilitate our understanding of the relative advantages/disadvantages of these various approaches.

FUTURE DIRECTIONS

- The experimental data are currently being analyzed by the interaction-based Hazard Index, which may lead to increased use of interaction data in component-based assessment of mixtures risk, and by proportional-response addition, a method of significant potential utility as it does not require mode-of-action assumptions.
- Experimental designs/analyses/techniques for evaluation of interactions will be developed that don't require single-chemical data as such data are not always available and when available, are not always useful.
- Because many environmental mixtures are highly complex and contain many unknown/unidentified chemicals, methods and techniques that allow for estimation of the contribution of this unidentified fraction to the toxicity of the whole mixture will be developed.
- As many nonadditive interactions have been identified at dose levels that are high relative to environmental exposure levels, future research will include a focus on identification of interaction thresholds (within a fixed mixing ratio, the nonadditive effect NOAEL/LOAEL) in experimental animal models and estimation, by use of physiologically-based pharmacokinetic models, of the associated internal tissues doses. By use of an assumption that equivalent internal dose results in equivalent toxicity in both experimental animals and humans, the PBPK models will be scaled to humans and used to predict the external human exposure concentrations at which nonadditive toxicity might be expected.

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